TSH AND DBC ACTION ON cAMP-DEPENDENT PROTEIN KINASE ACTIVITIES IN CELL CULTURES OF HUMAN NON-TOXIC GOITERS

M. PAVLOVIC-HOURNAC, D. DELBAUFFE, R. OHAYON, P. WADELEUX+ and R. WINAND+
Unité Thyroïde, Inserm, 78, rue du Général Leclerc, 94270 Bicêtre, France and +*Instituté de Pathologie, CHU, 4000 Sart Tilman par Liège I, Belgium*

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1. Introduction

Thyroid glands from different species contain two forms of cAMP-dependent protein kinases (type I, type II), which differ in their sensitivity to the TSH stimulation. Modifications of the type II enzyme are much more rapid and more important than those of the type I, both in the presence of excess, or in the absence of TSH [1,2]. The higher sensitivity of the type II kinase to hormonal action was also observed in rat testes [3]; in other systems it was the type I enzyme which was found to be more sensitive [4,5].

Here protein kinase activities were studied in cultured cells isolated from diffuse non-toxic human goiters. These cells can be cultured without loosing their capacity to metabolize iodine, or to respond to TSH by an increase in the intracellular level of cAMP [6].

We show that the pattern of protein kinases in cultured cells is different from the pattern of intact tissue, and that the activation ratios of the enzymes are different in cells cultured in the presence of TSH, and in those cultured with DBC.

2. Material and methods

2.1. *Cell cultures*

Human diffuse non-toxic goiters were used immediately after operation and were dissociated by the method detailed in [7,11]. Cells 24×10^6/petri dish (diam. 10 cm) were cultured for 6 days with medium changes every 48 h. TSH or DBC were added from the start of the culture period at 0.05–25 mU/ml or 0.25 mM for TSH and DBC, respectively. At the end of the culture, media were removed, cells washed and scraped and pellets kept at −80°C until used.

2.2. *Preparation of cytosol proteins and determination of protein kinase activities*

Cells (from 5–10 dishes) were resuspended in 1.5–2 ml Tris–HCl buffer [1,2], sonicated and centrifuged at 50 000 rev./min for 2 h. The supernatants were collected and the protein kinase activities determined either in total cytosol proteins or after their fractionation by sucrose gradient ultracentrifugation [2]. Cytosol proteins ~ 600–800 μl were layered onto top a 12 ml linear sucrose gradient (11–22%) and centrifuged in a Spinco Rotor SW 40 Ti at 38 000 rev./min, 0°C for 41 h. After fractionation, the cAMP-dependent protein kinase activities were determined in aliquots of each fraction detailed in [2]. The cAMP-dependent activity studied here refers to the net activity, which was calculated as follows: activity measured in the presence of cAMP from which the cAMP-independent activity was subtracted (detailed in [2]).

2.3. *Other methods*

cAMP measurements, DNA determinations and ^{125}I uptake and organification were carried out as in [7,11].

Abbreviations: TSH, thyrotropin; DBC, dibutyryl cyclic AMP

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3. Results

3.1. *Sucrose gradient ultracentrifugation pattern of protein kinase in cultured cells*

After fractionation of cytosol proteins by sucrose gradient ultracentrifugation it was observed that the pattern of protein kinase activities in cultured cells isolated from diffuse non-toxic goiter is different from that of non-cultured tissue. In goiter tissue, the 2 types of protein kinases are present (fig.1a) and their pattern is similar to the one observed in hypostimulated rat glands [1,2]. In cultured cells, however, only type I enzyme was found regularly (fig.1b).

In order to see if the disappearance of the type II enzyme is due to the culture conditions or to an intrinsic property of goitrous tissue, we analyzed the enzyme pattern of cultured cells isolated from normal dog thyroid. The 2 cAMP-dependent protein kinases were present in the same way as in intact glands (fig.1c). It would seem, therefore, that the disappearance of type II enzyme in goiter cell cultures is due to the cell origin rather than to the culture conditions.

3.2. *TSH and DBC action on protein kinase activities*

The analysis of the activation ratio (−cAMP/+cAMP activity) in control cultures and in TSH-treated cells showed that the cAMP-dependent protein kinases are apparently not activated. In DBC-treated cells the activation ratios are very high, indicating that the dissociation of protein kinases is very important and in some experiments is even complete (table 1).

Sucrose gradient ultracentrifugation analysis confirmed these findings. The pattern of protein kinase activity was not modified in cultures after addition of TSH (fig.2a). However, when cells were cultured in the presence of DBC the enzyme was almost totally dissociated and the free catalytic subunit was present in the gradient (fig.2b). This apparently paradoxical result obtained with TSH (absence of dissociation)

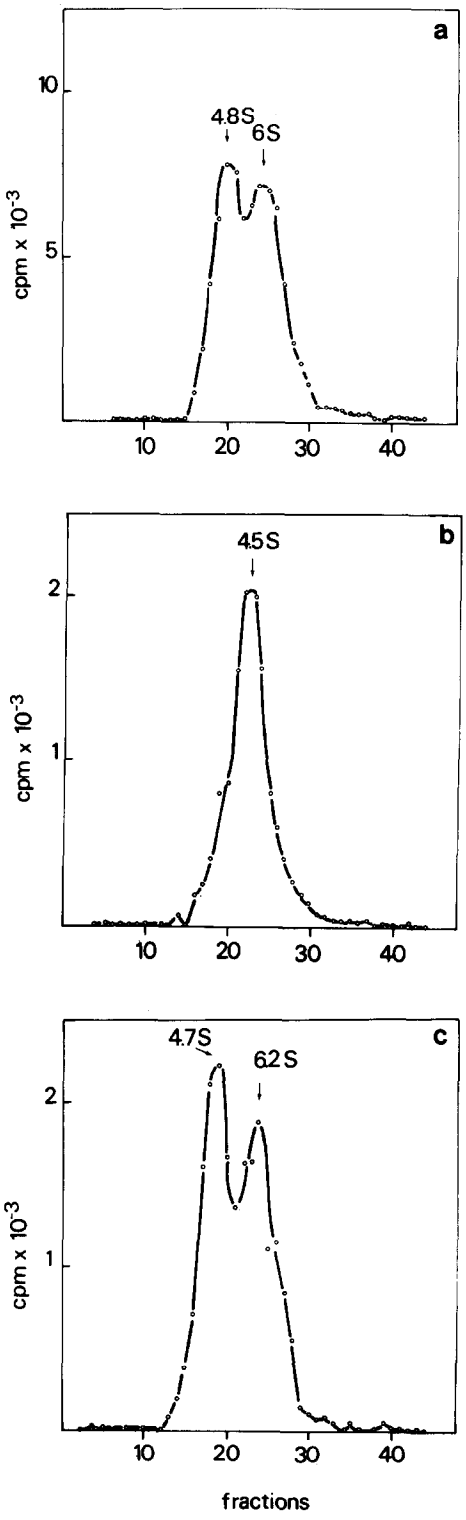

Fig.1. Sucrose gradient ultracentrifugation pattern of protein kinases in: (a) non-cultured goitrous tissue; (b) goiter cell culture; (c) dog cell culture. Experimental conditions are described in section 2. Results are expressed in cpm/μg DNA (cell cultures) or cpm/mg gland (non-cultured tissue).

Table 1

Activation ratios	$(\frac{-\text{cAMP}}{+\text{cAMP}}\text{activity})$
Controls	0.05 ± 0.02 (6)[a]
TSH-treated cells	0.03 ± 0.02 (5)
DBC-treated cells	0.56 ± 0.13 (5)

[a] No. determinations

suggests that the stability of enzyme activation may be different in the presence of the hormone or its mediator (cAMP) than in the presence of DBC. We therefore compared the stability of rat cytosol kinases after dissociation either by cAMP or DBC. It was observed that the dissociation of enzymes was reversible in the presence of cAMP while DBC dissociated the kinases permanently. In the TSH-treated cells the same phenomenon could occur either during the culture or during the separation of cytosol enzymes.

3.3. *TSH and DBC action on cAMP level and iodine organification*

The capacities of TSH and DBC to increase the intracellular level of cAMP and to stimulate the iodine organification were estimated in simultaneous cultures. The basal level of cAMP was similar to that obtained in cultures from normal human glands [6], in the presence of either TSH or DBC this level increased ~ 2-fold (table 2). No differences were observed between TSH- and DBC-treated cells.

The organification of iodine, evaluated in 2 different experiments, was increased when cells were

Table 2
TSH and DBC action on the cAMP level
(pmol cAMP/mg protein)

Control cells	TSH-treated cells	DBC-treated cells
6467 ± 203 (3)[b]	11 000 ± 1450[a] (3)	11 533 ± 1525[a] (3)

[a] $P < 0.005$
[b] No. experiments

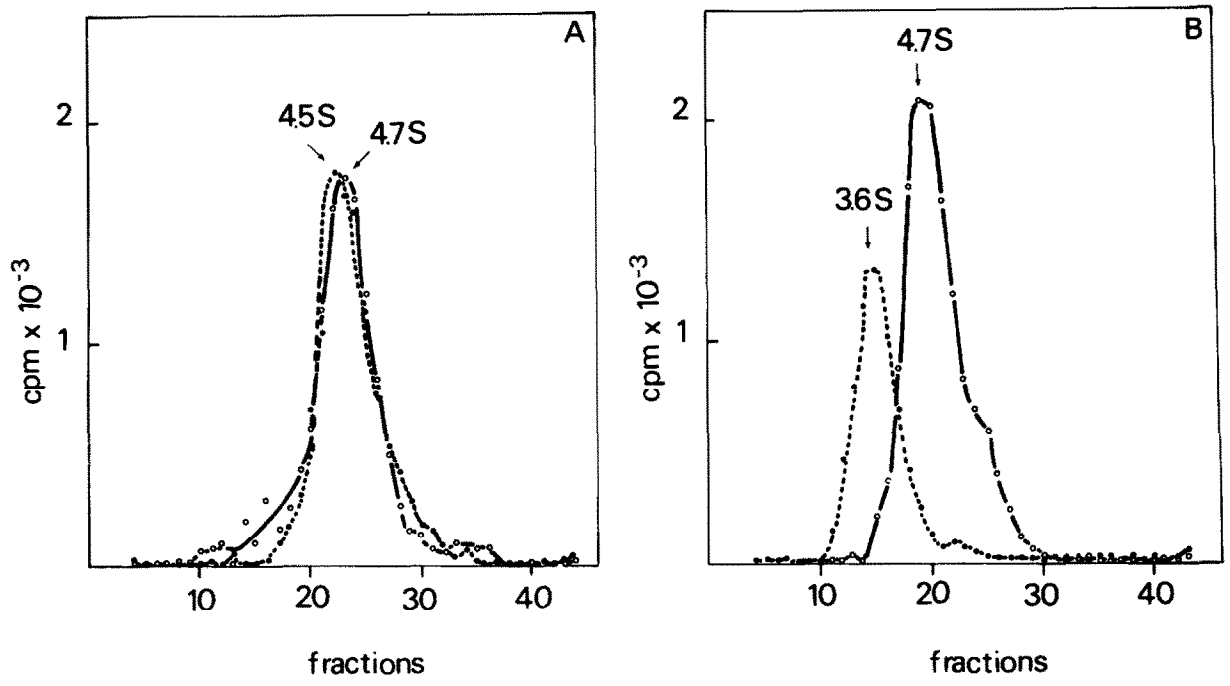

Fig.2. Sucrose gradient ultracentrifugation pattern of protein kinases in: (A) TSH-treated (●——●); (B) DBC-treated (●– – –●) cells, with their respective controls (○——○). Results are expressed in cpm/μg DNA.

Table 3
TSH and DBC action on iodine organification (cpm/mg DNA)

	Control cells	TSH-treated cells	DBC-treated cells
Expt. I	7000	14 000	17 000
Expt. II	19 000	34 000	32 000

cultured in the presence of TSH or DBC (table 3). It should be pointed out however, that both the basal and the stimulated levels of iodine organification in these cultures were extremely low when compared with values obtained with dog cells [7].

It was further observed, that neither TSH nor DBC can stimulate the uptake of iodine [8], nor induce the reassociation of cultured cells into follicular like structures, as is the case with dissociated porcine [9,10] or dog thyroid glands [11].

4. Discussion

Three main points emerge from this study. The modification of the enzyme pattern in cultured cells seems to be characteristic of the goiterous tissue; it did not occur in normal dog cell cultures. The disappearance of the type II enzyme provides further evidence to suggest that this enzyme entity is much more sensitive than the type I kinase in thyroid gland [1,2]. Its fragility in goiterous tissue could still be potentiated. It is however possible that pathological tissues may require different conditions of culturing for the maintenance of the fully differentiated state.

The fact that the organification of iodine takes place, and can be stimulated by both TSH and DBC even in the complete absence of the type II kinase, strengthens our hypothesis that the 2 isoenzymes of cAMP-dependent protein kinases may not have the same importance in the regulation of iodine organification [2]. More direct findings are still needed, however.

Our results also showed that the activation ratios of protein kinases in TSH-treated and DBC-treated cells are different. The absence of enzyme activation observed in TSH-treated cells is similar to the response obtained recently in chronically-stimulated rat glands [12]. During the long acting action of the hormone, as in the case of cell culture, the mechanism which is able to reverse the activated state of protein kinases may develop. In DBC-treated cells the activation of kinases appears to be much more stable. Therefore, in spite of its ability to mimic the hormone, DBC does not seem to be its true physiological substitute.

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